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CYTOGENETIC OBSERVATIONS OF NICKEL-INDUCED TUMORS IN MICE
[Observatii citogenetice asupra tumorilor induse cu nichel la sobolani]

by

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A cytogenetic study was carried out on 6 rat tumors grouped into 2 tumoral lines: NA and NB. The NA tumors represented different generations of a nickel-induced tumor transplant. With the NB line only the primary nickel-induced tumor was studied. Each line exhibited chromosomal particularities, characterized by the presence of special marker chromosomes (long submetacentric chromosome in the first line, a subtelocentric and a long telocentric chromosome in the second). In the course of transplantations the NA tumors showed a tendency to accumulation of polyploid cells; in another tumor belonging to this line disomia of the marker chromosome was noted. The likely significance of these findings is discussed.

Nickel
Mouse
Cytogenetic
Transplant
Chromosome
Tumor

In recent years the carcinogenic properties of metals have been the object of much study [14, 15] in an attempt to elucidate the details of the mechanism of carcinogenesis. Metal-induced rhabdomyosarcomas in mice have been analyzed from

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the biochemical [6, 7], histologic [4, 5], and electron microscopic [3] points of view. Krenova et al. [10] studied the cytogenetic and immunological characteristics of tumors induced with Spofa ferridextran in mice after prolonged cultivation in vitro.

In what follows we present some cytogenetic observations on nickel-induced tumors in mice.

Methods and Materials

In order to obtain tumors, three-month-old male Wistar mice were injected in their thigh muscles with 28 mg of nickel suspended in 0.4 ml of horse serum. Using this technique for a period of 3 - 5 months, we obtained tumors with a mean diameter of 2 - 3 cm.

With a view to transplantation, the animals were anaesthetized with ether and tumor fragments were gathered aseptically and inoculated subcutaneously with the aid of a trocar. The donor animals were then injected intraperitonially with colchicine in doses of 3 µg/g of body weight. The animals were sacrificed after 1½ hours and the tumors were shredded until a suspension was obtained. The cells were hypotonified in calf serum and distilled water in a ratio of 1:6 for 30 minutes. After fixation in methanol and glacial acetic acid in a ratio of 3:1, slides were made and stained by the method of Giemsa.

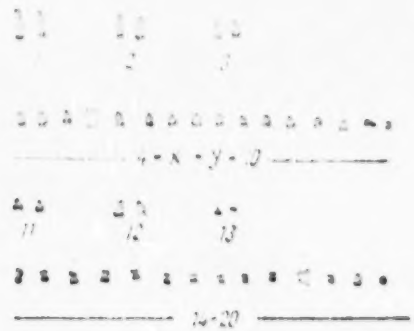

Fig. 1. Karyotype of male Wistar mouse

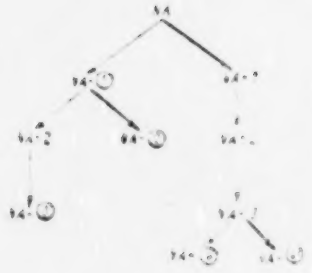

Fig. 2. Transplantation schema. Encircled tumors were studied from the cytogenetic point of view.

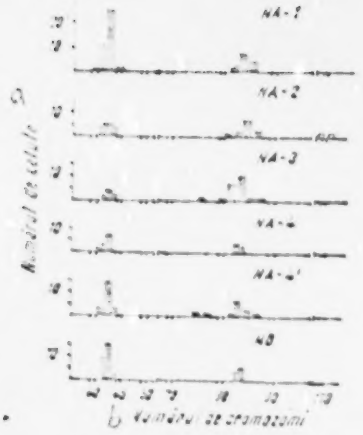

Fig. 3. Histogram showing chromosome distribution in NA and NB tumor lines.

Key: a. number of cells
b. number of chromosomes

The chromosomes were arranged according to the scheme of Hungerford and Nowell [9], Nowell et al. [11], and Ress et al. [13]. The karyotype of the male Wistar mouse utilized in this experiment is shown in Fig. 1.

The transplantation scheme is shown in Fig. 2. As can be seen from this schema, six tumors derived from the NA and NB tumor lines were studied. Five tumors of the NA line were examined: one each of the first (NA - 1), the second (NA - 2), and the third (NA - 3) transplant generation and two of the fourth (NA - 4 and NA - 4') transplant generation. Of the NB line, only the primary tumor was studied.

Results

Tumor line NA. All the tumors belonging to the NA line evinced two major distribution classes of tumoral cells: a hyperdiploid class and a hypertetraploid class. A minor octoploid class was identified in the NA - 2 tumor. The relationship between the hyperdiploid and hypertetraploid cells, however, is variable

(Fig. 3). In the case of the NA - 1 and NA - 3 tumors, the proportion of hypertetraploid cells amounts to 31.4% and 78.4%, respectively. The NA - 2 tumor evinced 40.6% of hypertetraploid cells, while the NA - 4 and NA - 4' tumors evinced 33.3% and 40.5%, respectively.

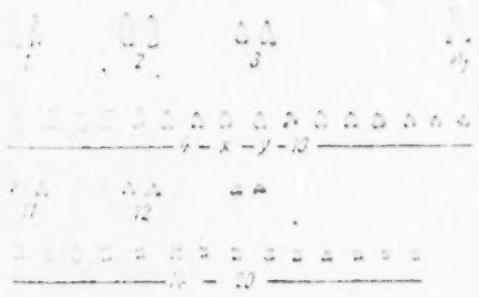

Fig. 4. Karyotype of a hyperdiploid NA cell. A marker chromosome (M_1) can be observed. This karyotype is characteristic of tumors NA - 1, NA - 3, NA - 4, and NA - 4'.

Fig. 5. Karytoype of a hyperdiploid NA cell. Two marker chromosomes (M_1, M_1) can be observed.

In tumors NA - 1, NA - 3, NA - 4, and NA - 4' a modal number of 43 chromosomes was observed. The supranumerary chromosome is submetacentric and somewhat longer than the chromosome of pair 1 (Fig. 4). In the hypertetraploid cells coming from this tumor, the presence of two marker chromosomes was observed. (Fig. 5).

The NA tumor evinced two marker chromosomes that are identical with the previously described chromosome in 29.4% of the hyperdiploid cells (Fig. 5). Twenty percent of the hypertetraploid cells evinced 3 - 4 similar chromosomes (Fig. 6, 7).

Tumoral line NB. In the NB tumor, we observed a similar distribution of cells belonging to the hyperdiploid and hypertetraploid classes (Fig. 3). The modal cells, with 43 chromosomes, evinced two marker chromosomes: a long subtelo-

centric one and a telocentric one whose length exceeds that of the number 1 chromosome; one of the number 13 chromosomes is lacking (Fig. 8).

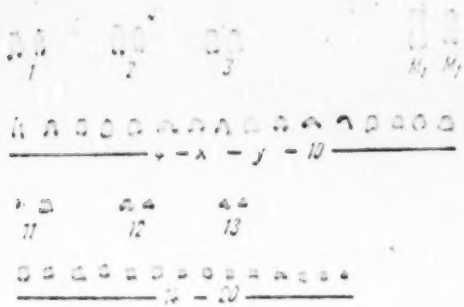

Fig. 6. Karyotype of a hyperdiploid NA - 2 cell. Two marker chromosomes (M_1, M_1) can be observed.

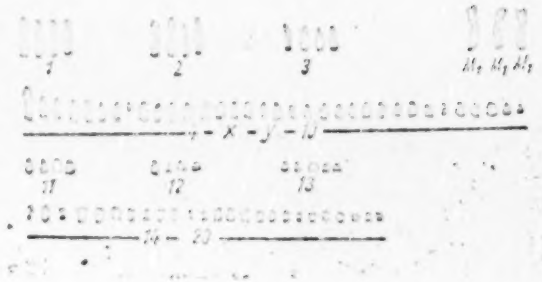

Fig. 7. Karyotype of a hypertetraploid NA - 2 cell. Three marker chromosomes of type M_1 can be observed.

Fig. 8. Karyotype of a hyperdiploid NB cell. Two marker chromosomes (M_1 and M_2) can be observed; one of the pair 13 chromosomes is absent.

Discussion

Swierenga and Basrur [17] have shown that nickel causes changes in the mitotic spindle: this might explain the unequal distribution of chromosomes in the course of mitosis. Heath and Webb [7] have observed that in the case of metal-induced rhabdomyosarcomas the ADN as well as ARN of the primary tumors contains cations belonging to the inducing metals. Interaction between the metal ions and nucleic acids might explain the chromosome aberrations.

The tendency of the polyploid cells in the NA - 3 tumor to accumulate as compared with the NA - 1 tumor suggests a modality of transformation similar to that described by Hsu et al. [8].

Karyotypical analysis of the hypertetraploid cells suggests that they arise from polyploidization of hyperploid cells and supports the hypothesis according to which the majority of tumors derives from a single abnormal cell [2].

An interesting fact is the doubling of the number of marker chromosomes of the NA - 2 tumor as compared with the other tumors belonging to the NA line. Pedersen [12] has observed a tendency of the Philadelphia chromosome toward mitotic nondisjunction, and we believe that the same phenomenon comes into play in the case of the marker chromosome described in the NA tumoral line.

The significance of the chromosome modifications observed in nickel-induced tumors has not been established: they might have a causal importance in the process of carcinogenesis, or might be simply a consequence of the malignant condition [1, 16]. Observations carried out on cultures of muscular tissue might contribute to elucidation of this problem. Be that as it may, the fact that once they have appeared, chromosome aberrations persist in the course of many transplant generations, demonstrates that they play an important role in the maintenance or progression of the neoplastic state.

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